Application Note 02033

Identification and Characterization of a Novel Delivery Molecule for a Clandestine PDE-5 Inhibitor Drug in a Dietary Supplement

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Introduction

Currently, there are three drugs on the market that are listed as approved by the U.S. Food and Drug Administration (FDA) to treat erectile dysfunction (ED): sildenafil (Viagra®), vardenafil (Levitra®), and tadalafil (Cialis®). These drugs are synthetic compounds that work by inhibiting the phosphodiesterase type 5 enzyme [1] and are commonly referred to as PDE-5 inhibitors. Estimated demand for ED treatment in China exceeds 300 million men. This has driven the demand for Traditional Chinese Medicine (TCM) ED treatments (i.e. herbal dietary supplements) combined with the push for more potent and effective analogues.

The three approved drugs are often the only PDE-5 inhibitor compounds that most forensic and contract labs check for in dietary supplements. However, some 28 or so analogues of these approved ED drugs are known to exist and can be easily overlooked [2]. As labs begin to develop methods for detecting the analogues, clandestine manufacturers will find ways to work around these detection methods by either: a) making new analogues of analogues, and/or b) making prodrug forms of analogues. A prodrug is a pharmacological substance (drug) that is administered in an inactive (or significantly less active) form. Once administered, the prodrug is metabolized in vivo into an active metabolite.

In this study, an herbal dietary supplement is investigated for the potential presence of synthetic PDE-5 inhibitors. A variety of analytical techniques are applied that allow the screening and characterization of the sample. Such techniques include LC/MS/MS, high-resolution mass spectrometry, and nuclear magnetic resonance spectroscopy (NMR). As a leader in analytical instrumentation technologies, Varian has a broad portfolio of products to help forensic scientists find answers in challenging situations.

Results and Discussion

Flora Research Laboratories in Grants Pass, OR received a dietary supplement for analysis. The manufacturer claimed that the supplement provided a cure for ED and was an all-natural product. The sample was a light yellow finely ground powder.

The laboratory had to ask these questions from a quality control standpoint before analysis:

1. How do you confirm that what you are seeing is actually adulteration from a PDE-5 inhibitor analogue and not a phytochemical (or naturally occurring) compound?
2. How do you test for a chemical compound you do not even know exists?
3. With so many analogues producing photo diode array (PDA) signatures matching the parent drug, how does one determine if they have an analogue, parent drug or prodrug?

It was determined that a quick way to screen the sample would be to use the Varian 500-MS ion trap LC/MS/MS. Figure 1 shows the complete 500-MS system used for the analysis at Flora Research Laboratories.

Figure 1. Varian 500-MS with 212-LC solvent delivery modules, 430 autosampler, and 335 diode array detector (PDA) at Flora Research Laboratories.

Typically, samples are run in MS/MS mode on either triple quadrupole or ion trap instruments. The 500-MS ion trap has the unique advantage to search for the presence of unknown compounds using the TurboDDS™ (Data Dependent Scanning) feature. It operates by performing a survey scan, and will automatically trigger MS^n experiments if an unknown contaminant is above a given instrument threshold. By expanding the mass range and using TurboDDS with up to MS^4 experiments, one can find designer drugs that may escape other detection methods.

When the sample was analyzed by TurboDDS™, the Figure 2 chromatogram and spectrum were detected.

As can be seen from the survey scan, the total ion chromatogram (TIC) shows only one major peak. Also, the PDA showed one major peak as well, indicating that the sample was relatively pure, which is not typical of a true dietary supplement containing complex phytochemical compounds. An ion tree report was created to display the data up through MS^4 as shown in Figure 3.

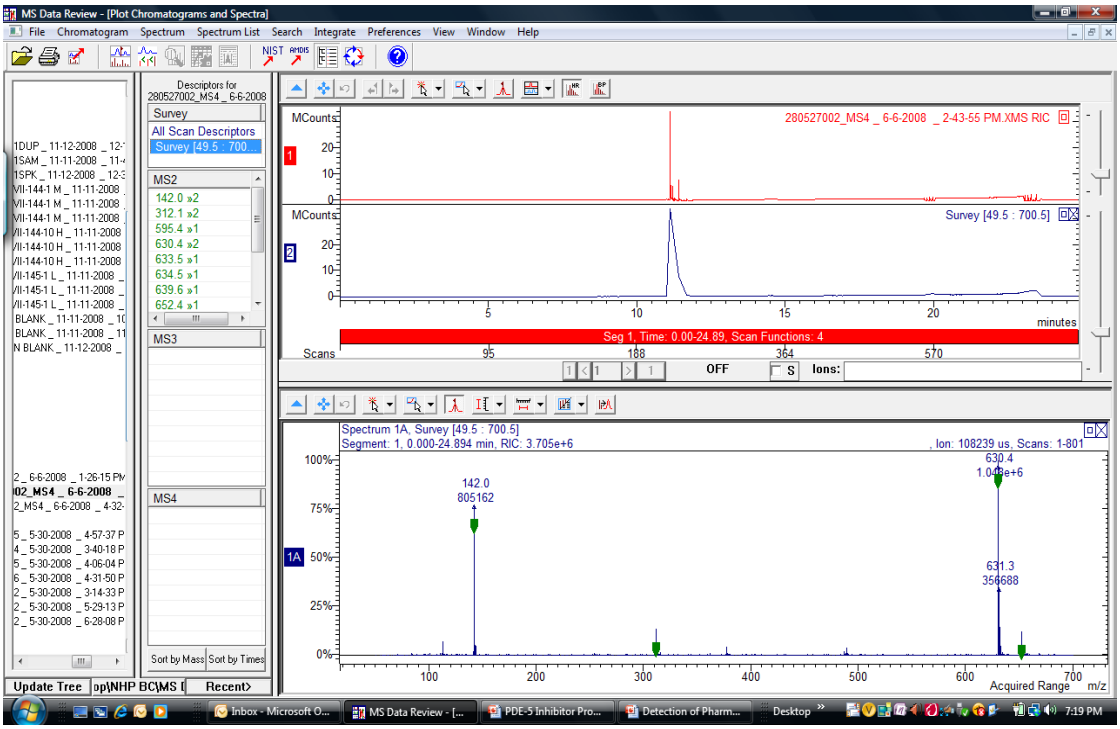

Figure 2. Survey scan from *m/z* 49.5-700.5 (top pane), and resulting MS/MS spectrum (lower pane). Major ion detected at M+H 630.4.

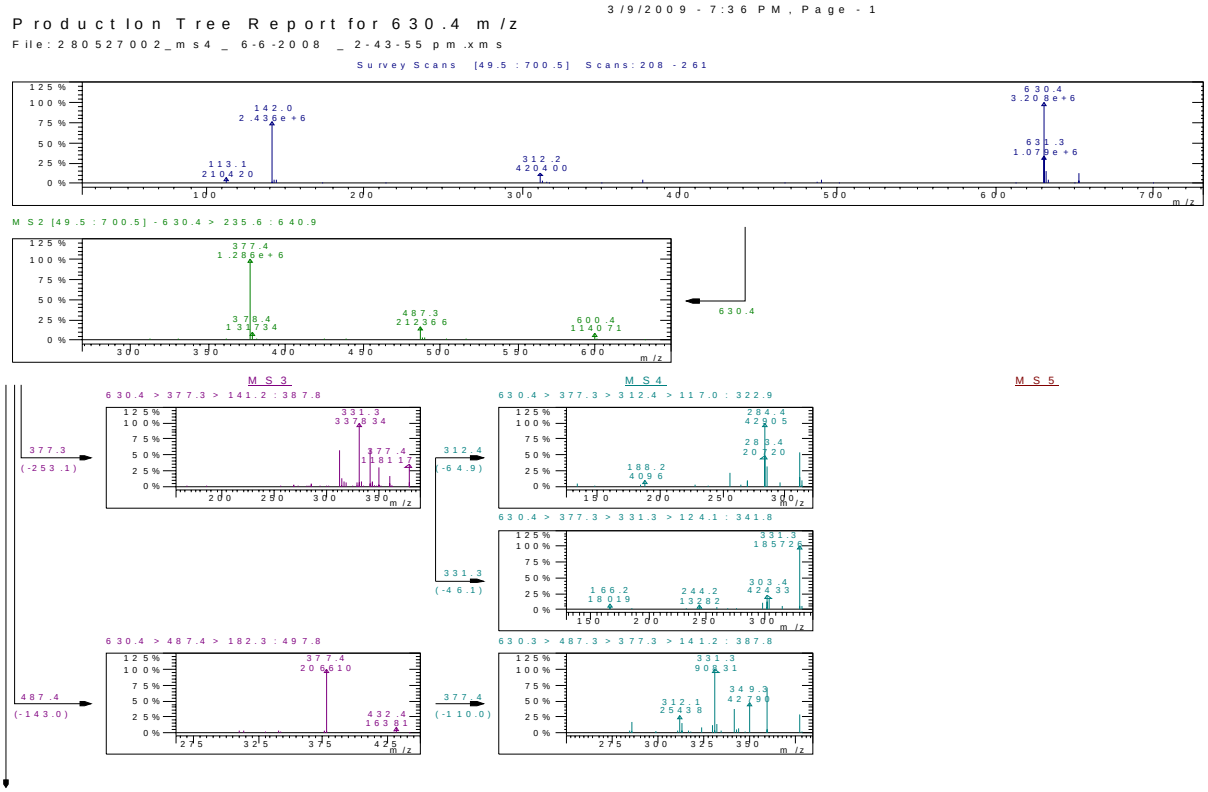

Figure 3. Product ion tree report of M+H *m/z* 630.4, from Varian MS Workstation software.

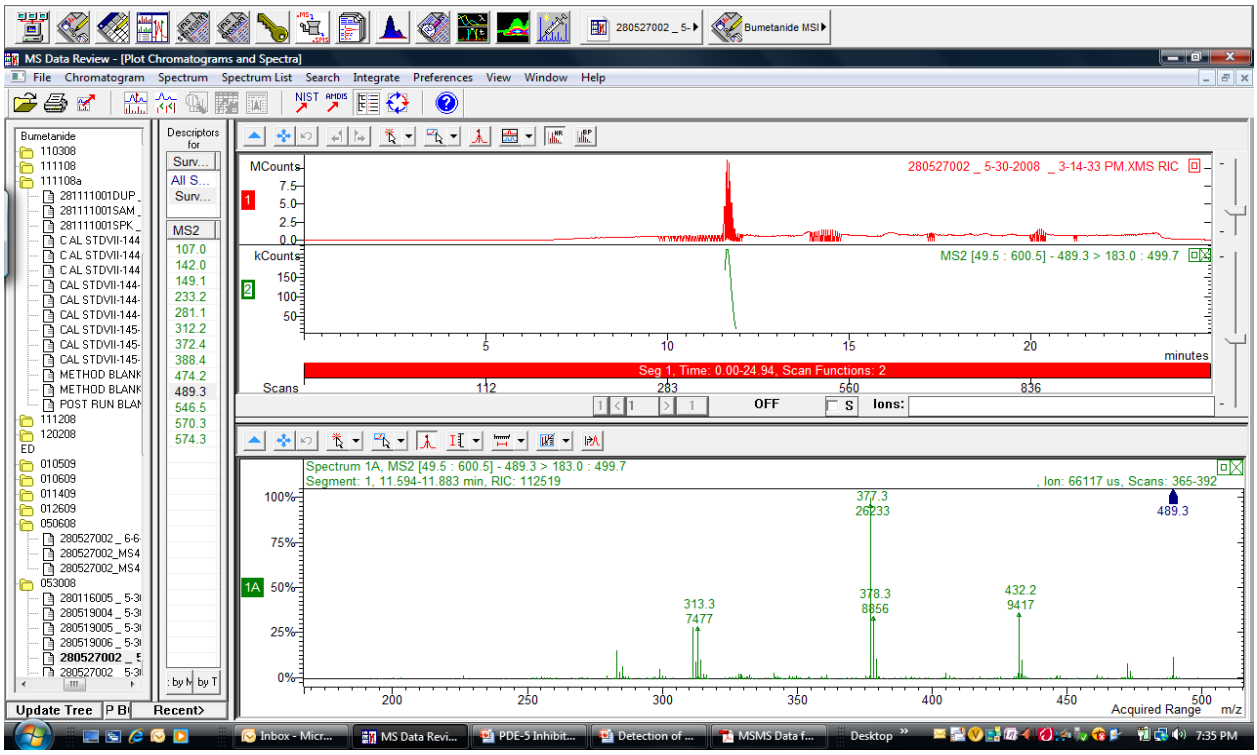

Figure 4. TurboDDS™ MS^2 product ion spectrum of *m/z* 489 with excellent match to aildenafil, a known PDE-5 analogue.

This data suggested that the compound might be related to aildenafil (a PDE-5 analogue). This led to the set-up of a second MS/MS experiment to look at *m/z* 489, known to be associated with aildenafil. Figure 4 shows the chromatogram with MS/MS spectral results.

It was concluded that the molecule definitely contained aildenafil (Figure 5), and the FDA confirmed this finding. In order to gain more structural information on this molecule, high-resolution FT-MS and NMR data were needed.

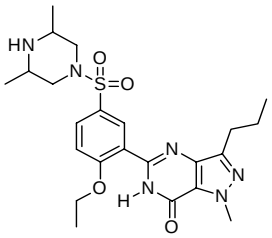

Figure 5. Structure of PDE-5 inhibitor analogue aildenafil.

FT-MS Data

A sample of the dietary supplement was sent to Varian, Lake Forest to obtain an exact mass measurement and potential empirical formula. Figure 6 is an exact mass spectrum taken by FT-MS for the compound. It indicates an exact mass of 630.22820. The exact mass for an aildenafil-like analogue was also confirmed with a collision induced dissociation (CID) experiment to yield a fragment at *m/z* 487.21188.

From this data a possible empirical formula was generated for the prodrug compound with mass accuracy near 1 ppm: $C_{27}H_{36}N_9O_5S_2$+1 with a 1.06 ppm mass error and a theoretical *m/z* of 630.22753.

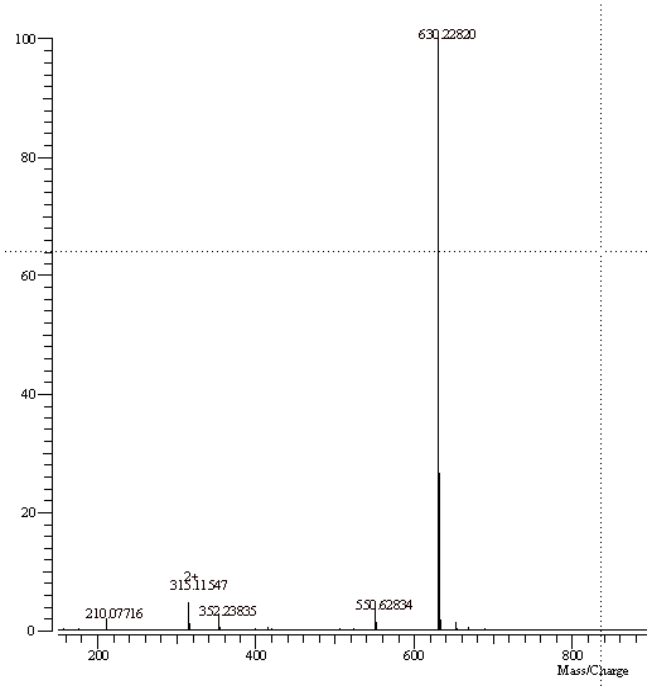

Figure 6. FT-MS exact mass spectrum of unknown prodrug.

NMR Data

NMR data sets were collected in an effort to gain more structural information. One-dimensional ^{1}H and ^{13}C spectra, as well as COSY (COrrelation SpectroscopY), HSQC (Heteronuclear Single Quantum Coherence), HMBC (Heteronuclear Multiple Bond Correlation), and ^{15}N- CIGAR (Constant time Inverse-detection Gradient Accordion Rescaled HMBC) NMR data sets provided very strong support for the presence of an aildenafil-like structural moiety in this sample, consistent with the MS data. An HMBC data set provides a proposed structural fragment: A five-membered imidazole ring where Q represents a heteroatom. This proposed fragment is shown in Figure 7.

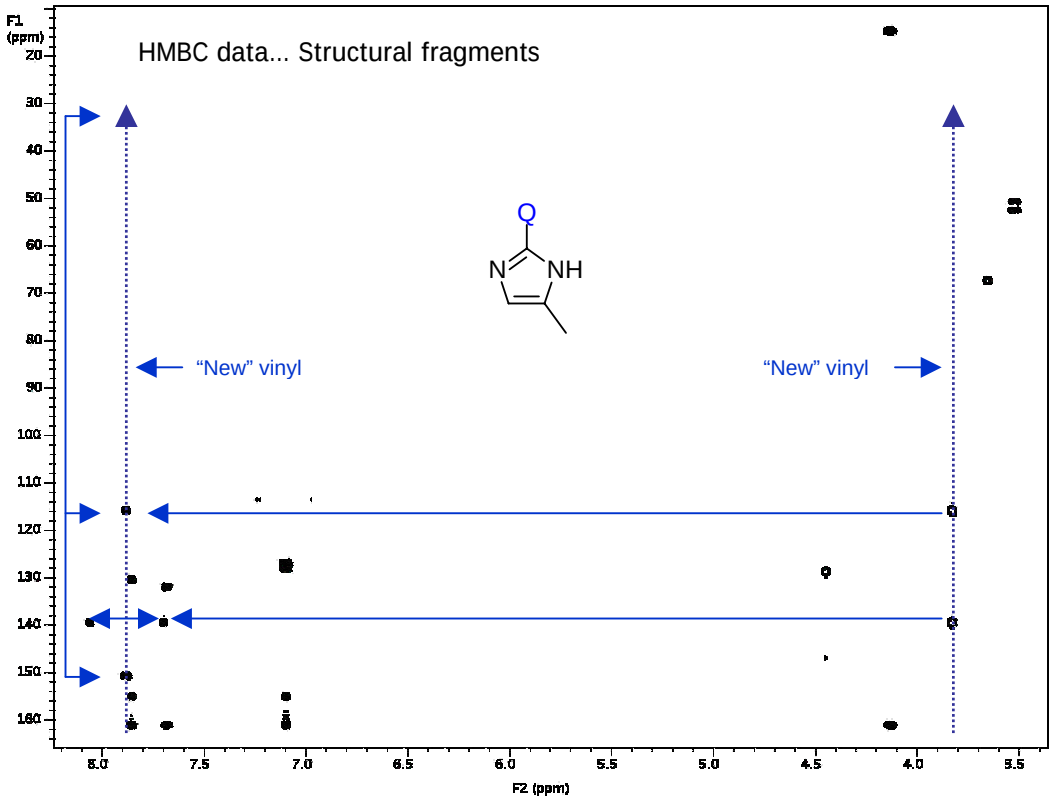

Figure 7. HMBC data set of a proposed structural fragment that is attached to the aildenafil analogue core.

Conclusion

The high demand for effective ED drugs has increased manufacture and sale of so called "natural" herbal dietary supplements. Clandestine manufactures are attempting to use PDE-5 analogues, analogues of analogues, or prodrugs of the big three blockbuster drugs to disguise adulteration of herbal dietary supplements.

Flora Research Laboratories received a so-called natural dietary supplement sample for analysis. The Varian 500-MS LC ion trap provided a rapid screen for unknown target compounds using the TurboDDS™ feature; it was immediately determined that the supplement sample contained an aildenafil-like analogue or prodrug. Other powerful techniques with Varian's broad scope of instrumentation, such as high resolution FT-MS and NMR, provided more structural information about the prodrug molecule.

The molecule itself is definitely related to the PDE-5 analogue aildenafil in its core structure, and has been modified with the addition of a 5-membered heterocyclic moiety. Its empirical formula to 1 ppm mass accuracy is $C_{27}H_{36}N_9O_5S_2$. The exact structure is yet to be determined.. Although the major pieces of the molecule have been characterized, further work is needed to put together the final proposed structure and will be presented in the next version of this application note.

The problem of dietary supplement adulteration with synthetic compounds is growing rapidly with new ones being discovered at an alarming rate.

References

1. Structural Elucidation of a PDE-5 Inhibitor detected as an adulterant in a Health Supplement, Xiaowei Ge, et al., *Journal of Pharmaceutical and Biomedical Analysis*, 48 (2008) 1070-1075.
2. See http://www.foodsafety.gov/~dms/supplmnt.html for more general information on dietary supplement regulations.

These data represent typical results.
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